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## **Heterocyclic aromatic amine [HCA] intake and prostate cancer risk: effect modification by genetic variants**

Van Hemelrijck, Mieke ; Rohrmann, Sabine ; Steinbrecher, Astrid ; Kaaks, Rudolf ; Teucher, Birgit ; Linseisen, Jakob

**Abstract:** The association between heterocyclic aromatic amine (HCA) intake and prostate cancer (PCa) risk may be modified by genetic variation in enzymes involved in HCA metabolism. We examined this question in a case-control study nested within the European Prospective Investigation into Cancer and Nutrition Heidelberg cohort. The study included 204 PCa cases and 360 matched controls. At baseline, participants provided dietary and lifestyle data and blood samples that were used for genotyping. Dietary HCA intake-2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), and 2-amino-3,4,8-dimethylimidazo [4,5-f]quinoxaline (DiMeIQx)-was estimated using information on meat consumption, cooking methods, and browning degree. Risk estimates for gene  $\times$  HCA interactions were calculated by unconditional logistic regression. We found inverse associations between PhIP, MeIQx, or DiMeIQx intake and PCa risk when having  $<2$  deletions of the GSTT1 and GSTM1 genes (P(interaction): 0.03, 0.01, and 0.03, respectively), which is supported by analysis of darkly browned meat consumption data. Statistically significant effect modification of both HCA (DiMeIQx) and darkly browned meat intake and PCa risk was observed for allelic variants of MnSOD (rs4880) (P(interaction): 0.02). Despite limitations due to study size, we conclude that the association between HCA intake and PCa risk could be modified by polymorphisms of GSTT1, GSTM1, and MnSOD.

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# **HCA intake and prostate cancer risk: effect modification by genetic variants**

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## Abstract

The association between heterocyclic aromatic amine (HCA) intake and prostate cancer (PCa) risk may be modified by genetic variation in enzymes involved in HCA metabolism. We examined this question in a case-control study nested within the European Prospective Investigation into Cancer and Nutrition-Heidelberg cohort. The study included 204 PCa cases and 360 matched controls. At baseline, participants provided dietary and lifestyle data and blood samples which were used for genotyping. Dietary HCA intake (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), and 2-amino-3,4,8-dimethylimidazo [4,5-f]quinoxaline (DiMeIQx) was estimated using information on meat consumption, cooking methods, and browning degree. Risk estimates for gene\*HCA interactions were calculated by unconditional logistic regression. We found inverse associations between PhIP, MeIQx, or DiMeIQx intake and PCa risk when having <2 deletions of the *GSTT1* and *GSTM1* genes ( $P_{\text{interaction}}$ : 0.03, 0.01, and 0.03, respectively), which is supported by analysis of darkly browned meat consumption data. Statistically significant effect modification of both HCA (DiMeIQx) and darkly browned meat intake and PCa risk was observed for allelic variants of *MnSOD* (rs4880) ( $P_{\text{interaction}}$ : 0.02). Despite limitations due to study size, we conclude that the association between HCA intake and PCa risk could be modified by polymorphisms of *GSTT1*, *GSTM1*, and *MnSOD*.

## Introduction

A western diet has long been considered as a potential risk factor for prostate cancer (PCa). In the context of meat consumption, evidence is weak for an association between both red and processed meat intake and PCa risk <sup>1-2</sup>. However, the intake of grilled meat is thought to be related to PCa risk since high-temperature cooking of meat leads to formation of mutagenic heterocyclic aromatic amines (HCA), which have been shown to induce tumours in experimental animal models <sup>3-4</sup>. Cooking at higher temperatures and for longer periods of time both result in formation of more HCA <sup>3-4</sup>. To date, several observational studies have evaluated the association of intake of meat cooked at high temperature and PCa risk, but results are inconsistent <sup>5-9</sup>.

The association between PCa risk and intake of the three most mass-abundant HCAs detected in cooked meat, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), and 2-amino-3,4,8-dimethylimidazo [4,5-f]quinoxaline (DiMeIQx), was also studied in our European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort. Even though meat consumption, cooking methods, and degree of browning of the respective food items were assessed with a detailed questionnaire, the results did not indicate that HCA intake, as consumed in a regular diet, was associated with PCa risk <sup>10-11</sup>. However, experimental studies have shown that levels of PhIP approximating human dietary exposure stimulate cellular signalling pathways and result in increased growth and migration, suggesting a link with the promotion and progression of neoplastic disease <sup>4, 12</sup>. One of the reasons for our null-findings might be that the association between HCA intake and PCa risk is modified by different genotypes/polymorphisms in genes encoding for HCA-metabolizing enzymes.

So far, only two studies investigated such gene-diet interactions on PCa risk<sup>13-14</sup>, but evidence has been found for other cancers such as the GST status-diet interaction in the context of lung cancer<sup>15</sup>. Therefore, we aimed to assess interactive effects of polymorphisms of genes involved in the metabolism of HCA on the association between HCA and PCa risk in a case-control study nested within the prospective EPIC-Heidelberg cohort.

## **Methods**

### *Study population*

Data was taken from the EPIC-Heidelberg study, an ongoing prospective cohort study assessing the association between dietary, lifestyle, and metabolic factors and risk of cancer. From 1994 to 1998, a total of 11,928 men aged 50-65 years and 13,612 women aged 35-65 years were recruited from the general population of Heidelberg and surrounding communities<sup>16</sup>. At baseline, dietary, lifestyle, medical and socioeconomic data were collected via self-administered questionnaires and personal interviews. The cohort is followed up by mailed questionnaires in intervals of about three years to assess information on health status, diet, and lifestyle<sup>17</sup>. Diet was assessed by using a validated food-frequency questionnaire (FFQ)<sup>18</sup>.

During the second follow-up of the cohort (2002-2004), 11,605 men were contacted; of these, 9,864 participants completed a 158-item FFQ. This FFQ included detailed questions on meat preparation methods and preferred degree of browning. Mean daily dietary intake of HCAs from meat was calculated by using published data on the HCA content of different types of meat in combination with information on degree of browning, cooking methods, and the amount of meat intake<sup>10</sup>. Details about the development of this short questionnaire to assess the dietary intake of HCA have been published elsewhere<sup>19</sup>.

Self-reported cases of PCa were verified based on medical reports by the study physician. Additionally, death certificates of deceased participants were checked for PCa as underlying cause of death. Information on stage and grade of PCa was extracted by the study physician from pathology reports, including tumour nodal metastasis (TNM) stage and Gleason histologic grade. Advanced PCa was defined as PCa with a Gleason score  $\geq 7$ , a TNM staging score of T3/T4, N1-N3, or M1, or PCa as the underlying cause of death.

For the present study, we used a nested case-control approach based on all male EPIC-Heidelberg participants with blood samples available and free of prevalent cancer (except nonmelanoma skin cancer) at baseline. All incident PCa cases (C61, C63.8 and C63.9; *International Classification of Disease for Oncology, Second Edition*) diagnosed by end of February 2007 were selected. Following an incidence density sampling protocol, two controls were matched per case by age (5-year age groups) and time of recruitment (6-months intervals). After excluding those with no information on HCA intake, the final study population comprised 203 cases and 360 controls.

All participants gave written informed consent and the study was approved by the ethics committee of the Heidelberg Medical School.

#### *Laboratory Analyses*

Genomic DNA was extracted from buffy coat with FlexiGene kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. DNA was stored at 4°C until use. To determine deletions of the *GSTM1* and *GSTT1* genes, a semi-quantitative genotyping assay on the LighCycler 480 (Roche, Mannheim, Germany) was used, with multiplexing of both

genes using albumin as reference gene and internal control to confirm amplification<sup>20</sup>. This method allows for the distinction of homozygous, heterozygous, and non-carriers. Determination was done in triplicated and a SD of >10% led to repeated analysis. Five percent of the samples were repeated for quality-control reasons and concordance of the assigned genotypes was >95%. Genotyping for polymorphisms of the genes *NQO1* (C609T, rs1800566), *GSTA1* (G-52A, rs3957357), *GSTP1* (A313G, rs1695; C341T, rs1138272), *MnSOD* (C47T, rs4880), *CYP1A1* (3801T>C, rs4646903 [CYP1A1\*2A]; C1382A, rs1799814 [CYP1A1\*4]), *GPX1* (C>T, Pro198Leu, rs1050450), and *GPX4* (C718T, rs713041) were done as multiplex on the MassArray system (Sequenom, San Diego, USA) applying the iPLEX method and matrix-assisted laser desorption/ionization – time-of-flight mass spectrometry for analyte detection. The analysis was carried out by BioGlobe GmbH (Hamburg, Germany). All duplicated samples (quality-control repeats of 8% of the samples) to verify inter-experimental reproducibility and accuracy delivered concordant genotype results. The genotype could not be assigned to six samples for the *GPX1* single nucleotide polymorphism (SNP) and to three samples for the *GPX4* SNP. All laboratory analyses were carried out with the laboratory personnel blinded to the case-control status.

### *Statistical methods*

Baseline characteristics of the study population are given as mean and SD or percentages by case-control status. Median and interquartile range were computed for dietary intake data. Genotype frequencies of all studied gene polymorphisms are presented and the  $\chi^2$  test was used to check for Hardy-Weinberg equilibrium. The associations between those polymorphisms not previously studied in the context of PCa risk<sup>21-22</sup> were calculated with conditional logistic regression estimating odds ratios (OR) and 95% confidence intervals

(CI), with the most frequent variant being the reference category. The analysis was stratified by case set.

To evaluate potential effect modification of the association between HCA intake and PCa risk by genotype, we calculated OR (95%CI) of PCa for each HCA intake variable stratified by genotype with unconditional logistic regression adjusting for the matching variables (time of recruitment and 5-year age groups) and family history of PCa. Each HCA intake variable was also adjusted for daily energy intake (per 1000 kcal). Due to the small units of each HCA intake variable, we changed the units of each component as following: PhIP intake was based on 50 ng/1000kcal increments, whereas MeIQx intake and DiMeIQx were based on 10 ng/1000kcal and 1ng/1000kcal increments, respectively. All HCA intake variables were analyzed as continuous variables. Because of the small numbers in some genotype categories, we also combined the heterozygote and homozygote (mutant) categories. Furthermore, we combined *GSTM1* and *GSTT1* genotypes by counting the number of deleted alleles and grouping into zero to one, two, and three to four deleted alleles. We repeated the above analysis with additional adjustment for intake of red meat, processed meat, fruit and vegetables, and intake of glucosinolates. Statistical significance of the interaction between genotypes and HCA intake (cross-product) was assessed with the Wald-test. To verify whether other carcinogenic meat compounds (e.g. polycyclic aromatic hydrocarbons (PAH)) are involved in addition to HCA we repeated the above analyses for ‘meat intake by degree of browning’ (10 g/day increments of dark or light browned consumed meat) which may capture both HCA and PAH intake. All analyses were conducted with Statistical Analysis Systems (SAS) release 9.1.3 (SAS Institute, Cary, NC).

## Results



Baseline characteristics of the study population are presented in Table 1. Cases and controls did not differ with respect to age or BMI. Cases were less likely to be former or current smokers and had less often a university degree. More cases than controls reported a positive family history of PCa.

The associations between gene polymorphisms and prostate cancer risk (main effects) were reported earlier<sup>21-22</sup> and are thus not shown here. The allele frequencies of the selected genetic variants in PCa cases and controls are shown in Table 2. Among the newly tested genetic variants *GSTP1* (C341T, rs1138272), *MnSOD* (C47T, rs4880), and *CYP1A1* (3801T>C, rs4646903; C1382A, rs1799814)), the CYP1A1\*4 variant rs1799814 was found to be statistically significantly associated with PCa risk: men with a CA genotype were more likely (OR: 1.94, 95%CI: 1.08-3.48) to develop PCa compared to men with the wildtype. As reported earlier, no significant (main) effects of HCAs on PCa risk were found in this cohort<sup>11</sup>.

Next, we assessed how gene polymorphisms might affect the association between different types of HCA intake and PCa risk. From Table 3 it can be seen that there was an inverse association between intake of PhIP, MeIQx, or DiMeIQx and risk of PCa when having less than two deletions of the *GSTT1* and *GSTM1* genes ( $P_{\text{interaction}}$ : 0.03), though none of the OR were found to be statistically significant (e.g. OR when having <2 deletions for association between energy-adjusted PhIP intake (per 50ng/d) and PCa: 0.55 (95%CI: 0.30-1.02)). We also identified a statistically significant interaction between *MnSOD* (rs4880) and DiMeIQx intake on PCa risk ( $P_{\text{interaction}}$ : 0.02) such that homozygous carriers of the mutant allele may have a lower risk. Additionally, there was a statistically significant association between GPX4 (rs713041) and DiMeIQx intake on PCa risk ( $P_{\text{interaction}}$ : 0.03), indicating that the wild

type was at increased risk. Adjustment for intake of red meat, processed meat, fruit and vegetables, and intake of glucosinolates did not alter the results (results not shown).

Subsequently we tested the same associations and effect modifications for advanced PCa (results not shown). We only found a statistically significant effect modification by deletions of the *GSTT1* and *GSTM1* genes for the association between intake of energy-adjusted PhIP (per 50 ng/d) and risk of advanced PCa (OR<sub>2del</sub>: 0.51, 95%CI: 0.22-1.20; OR<sub>3-4del</sub>: 0.78, 95%CI: 0.55-1.11; P<sub>interaction</sub>: 0.03).

Finally, we studied how the association between 10 g/day increments of consumption of lightly and darkly prepared meat and risk of prostate cancer was modified by different gene polymorphisms (Table 4). Using the food-based data we could confirm allelic variants of MnSOD (rs4880) as possible effect modifiers (P<sub>interaction</sub>: 0.02) as already identified by means of DiMeIQx intake data. A statistically significant inverse association between consumption of darkly prepared meat and risk of prostate cancer existed in men with the TT genotype, while in men with the wild type or heterozygote form there was no association. In addition, we found indication for effect modification by variants of *GSTM1* and *T1*, statistically significant for men with no deletions in *GSTT1* and consumption of darkly browned meat (P<sub>interaction</sub>: 0.07).

## Discussion

In the current study, we examined effect modification of the association between dietary intake of the heterocyclic amines PhIP, MeIQx, and DiMeIQx, and PCa risk by polymorphisms in genes encoding for HCA-metabolizing enzymes. Our results showed inverse associations between intake of PhIP, MeIQx, or DiMeIQx and risk of PCa when

having less than two deletions of the *GSTT1* and *GSTM1* genes. A statistically significant interaction was also noted between DiMeIQx intake and the polymorphism rs4880 of the MnSOD locus ( $P_{\text{interaction}}$ : 0.02). These results were largely supported by using food-based data, i.e. differently prepared meat intake data, instead of HCA intake data.

Observational studies based on dietary questionnaires have found contradicting results for the association between meat cooking methods (or HCA intake) and risk of PCa. For instance, the NIH-AARP Diet and Health study, containing 10,313 PCa cases, assessed different types of meat-cooking methods and found that grilled/barbecued meats were related with an 11% risk increase of total PCa and a 36% risk increase of advanced PCa<sup>9</sup>. In contrast, the Agricultural Health Study, based on 57,311 men, did not find an association between specific cooking methods and PCa risk, but intake of well or very well done total meat was associated with a 1.26-fold increased PCa risk and a 1.97-fold increased risk of advanced disease<sup>8</sup>. In the EPIC-Heidelberg cohort, based on 9,578 men, dietary HCA intake was estimated using information on meat consumption, cooking methods, and preferred degree of browning, but the data also did not support the hypothesis that HCA intake as consumed in a regular diet is a risk factor for PCa<sup>11</sup>. The current study aimed to explain some of these contradictory findings by assessing genetic polymorphism and as a consequence inter-individual variability.

To date only two nested case-control studies investigated how risk of prostate cancer associated with dietary HCAs intake may be modified by single nucleotide polymorphisms (SNP) in genes involved in the HCA metabolism<sup>13-14</sup>. In the study based on the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial they studied 12 different genes (CYP1A1, CYP1A2, CYP1B1, GSTA1, GSTM1, GSTM3, GSTP1, NAT1, NAT2,

SULT1A1, SULT1A2, and UGT1A locus), whereas the study from the Multiethnic Cohort assessed NAT1 and NAT2 acetylator genotypes. The later one did not find evidence for an effect-modification by these genotypes <sup>13</sup>, but the study by Koutros and colleagues found evidence for effect-modification by polymorphisms of the GSTM3 locus <sup>14</sup>. Thus, HCA-gene interactions have not been studied extensively and need to be addressed in other studies with detailed information on HCA intake and SNPs.

Different enzymes are involved in the metabolism of HCA and hence functional polymorphisms of their genes may modify the association between HCA and risk of PCa. Heterocyclic amines require metabolic activation to exert their genotoxic effects which involves phase I hepatic cytochrome P450 (CYP)-mediated N-hydroxylation followed by phase II conjugation of the N-hydroxylamines to ester derivatives that may react with DNA <sup>23-24</sup>. N-acetyltransferases (NATs) and sulfotransferase (SULT) can then activate N-hydroxy-HCA, and more specifically an ATP-dependent pathway of activation has been identified to form PhIP in the prostate <sup>23, 25</sup>. As a consequence, increased expression and metabolic activation of *CYP1A1*, *CYP1A2* and *CYP1B1* has been shown in benign prostatic hyperplasia (BPH) tissue <sup>23, 26</sup>. In contrast, HCA carcinogens are known to be detoxified by glutathione S-transferases (GSTs) and possibly also NADPH-quinone oxidoreductase (NQO1) <sup>22-23, 25</sup>. Recently, polymorphisms of manganese superoxide dismutase (MnSOD), an enzyme involved in the protection from reactive oxygen species (ROS)-mediated DNA damage, were also found to be related with risk of PCa <sup>27</sup>. In the context of cellular oxidative stress, glutathione peroxidases (GPx) may be another type of enzymes involved in PCa risk as they are important components of the redox control system in humans <sup>21</sup>.

In our EPIC-Heidelberg cohort, we previously have shown that polymorphisms of *GSTM1*, *GSTT1*, *GSTP1*, *GSTA1*, *NQO1*, *GPX1*, and *GPX4* were not associated with PCa risk<sup>21-22</sup>. From the additional polymorphisms assessed in this study, the C/A substitution of *CYP1A1* (rs1799814) was the only polymorphism found to increase the risk of PCa considerably. To date, only one experimental study compared frequencies of *CYP1A1*\*4 (rs1799814) among PCa cases and unaffected men<sup>28</sup>, but did not find any statistically significant differences. In contrast, the study by Chang and colleagues found an association between polymorphisms of *CYP1A1*\*2A and *CYP1A1*\*2C and PCa risk<sup>28</sup>. We did not have information on *CYP1A1*\*2C in this study, but for *CYP1A1*\*2A we found no association.

We found that in participants with less than two deletions of the *GSTT1* and *GSTM1* genes, higher intake of PhIP, MeIQx, or DiMeIQx was associated with a lower risk of PCa overall compared to those with two or more deletions. This reflects the fact that subjects homozygous for a null allele are considered detoxification deficient<sup>29</sup> and thus less protected for the carcinogenic properties of HCA. In contrast, a lower risk of advanced PCa was found when having more than 2 deletions, which may suggest a different mechanism of action between HCA, the studied SNPs, and development versus progression of PCa. Given the existing literature, it is somewhat surprising to see a decreased risk instead of a less increased risk of PCa related to higher HCA intake<sup>30</sup>. However, the HCA-based results are confirmed to some degree by using meat consumption data.

The association between DiMeIQx intake and PCa risk was modified by a polymorphism of *MnSOD* (C47T). Participants with a homozygote mutant genotype (who have an amino acid change from valine (Val) to alanine (Ala)) had a decreased risk of PCa when having a higher DiMeIQx intake or a higher intake of darkly prepared meat. Subjects with the Ala/Ala

phenotype (i.e., CC coded) may have higher MnSOD activity compared to those with the Val/Val or Ala/Val phenotype <sup>31</sup>. A recent meta-analysis based on 8,962 subjects showed that those with Ala variant genotypes were at increased risk of PCa compared to those with the wild type <sup>31</sup>, which thus suggests that *MnSOD* as an endogenous antioxidant in mitochondria may play an important role in preventing PCa <sup>31</sup>. However, only one study investigated the *MnSOD* genotype in relation to HCA (due to smoking) and PCa risk and showed that there was only an association between *MnSOD* Ala/Ala genotype and PCa risk among rapid *NAT1* genotypes and smokers <sup>27</sup>.

A major strength of the present nested case-control study is its prospective design, the high follow-up rate (>90%), and the medically confirmed diagnoses of PCa. Furthermore, we were able to adjust our analyses with respect to known or suspected confounders, but residual confounding cannot be completely ruled out. Our sample size of 203 cases was rather small, but we improved study power by matching two controls per case. However, due to missing data on HCA some cases only had one control. Although we were able to determine some gene-diet interaction effects, we might not have had enough power to detect some associations with smaller effects. Moreover, we did not adjust for multiple testing. Thus, results have to be seen as a first indication for possibly relevant effect modification that has to be confirmed in larger studies, especially given the limited statistical significance of our findings. Another limitation of this study is the possible misclassification of HCA intake, even though the 2 major determinants (i.e., degree of browning and preparation method) were included in the photo-based questionnaire and we previously showed a positive association between HCA intake and risk of colorectal adenomas in the same cohort using the same dietary questionnaire <sup>32</sup>. We also lack data on other possible genotypes that might modify the association between HCA intake and PCa risk (e.g. *CYP1A1*\*2C or *NAT1*/2).

### *Conclusion*

This study indicates that the association between HCA intake and PCa risk – as well as consumption of darkly browned meat - is modified by polymorphisms in *GSTT1*, *GSTM1*, and *MnSOD*, however, these modifications are not necessarily concordant with the underlying biological hypotheses. Considering genetic variation is thus an important step in elucidating the mechanism of action between HCA or meat intake and risk of PCa. Other larger studies are needed to investigate the complex interplay of polymorphisms in genes encoding HCA-metabolizing enzymes and intake of different HCA.

## References

1. Zheng W, Lee SA. Well-done meat intake, heterocyclic amine exposure, and cancer risk. *Nutr Cancer*. 2009;61(4):437-446.
2. Alexander DD, Mink PJ, Cushing CA, Scurman B. A review and meta-analysis of prospective studies of red and processed meat intake and prostate cancer. *Nutr J*. 2010;9:50.
3. Tang D, Liu JJ, Rundle A, et al. Grilled meat consumption and PhIP-DNA adducts in prostate carcinogenesis. *Cancer Epidemiol Biomarkers Prev*. Apr 2007;16(4):803-808.
4. Creton SK, Zhu H, Gooderham NJ. The cooked meat carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine activates the extracellular signal regulated kinase mitogen-activated protein kinase pathway. *Cancer Res*. Dec 1 2007;67(23):11455-11462.
5. Cross AJ, Peters U, Kirsh VA, et al. A prospective study of meat and meat mutagens and prostate cancer risk. *Cancer Res*. Dec 15 2005;65(24):11779-11784.
6. Norrish AE, Ferguson LR, Knize MG, Felton JS, Sharpe SJ, Jackson RT. Heterocyclic amine content of cooked meat and risk of prostate cancer. *J Natl Cancer Inst*. Dec 1 1999;91(23):2038-2044.
7. Rovito PM, Jr., Morse PD, Spinek K, et al. Heterocyclic amines and genotype of N-acetyltransferases as risk factors for prostate cancer. *Prostate Cancer Prostatic Dis*. 2005;8(1):69-74.
8. Koutros S, Cross AJ, Sandler DP, et al. Meat and meat mutagens and risk of prostate cancer in the Agricultural Health Study. *Cancer Epidemiol Biomarkers Prev*. Jan 2008;17(1):80-87.
9. Sinha R, Park Y, Graubard BI, et al. Meat and meat-related compounds and risk of prostate cancer in a large prospective cohort study in the United States. *Am J Epidemiol*. Nov 1 2009;170(9):1165-1177.
10. Rohrmann S, Zoller D, Hermann S, Linseisen J. Intake of heterocyclic aromatic amines from meat in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort. *Br J Nutr*. Dec 2007;98(6):1112-1115.
11. Sander A, Linseisen J, Rohrmann S. Intake of heterocyclic aromatic amines and the risk of prostate cancer in the EPIC-Heidelberg cohort. *Cancer Causes Control*. Jan 2011;22(1):109-114.
12. Wang CY, Debiec-Rychter M, Schut HA, et al. N-Acetyltransferase expression and DNA binding of N-hydroxyheterocyclic amines in human prostate epithelium. *Carcinogenesis*. Aug 1999;20(8):1591-1595.
13. Koutros S, Berndt SI, Sinha R, et al. Xenobiotic metabolizing gene variants, dietary heterocyclic amine intake, and risk of prostate cancer. *Cancer Res*. Mar 1 2009;69(5):1877-1884.
14. Sharma S, Cao X, Wilkens LR, et al. Well-done meat consumption, NAT1 and NAT2 acetylator genotypes and prostate cancer risk: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev*. Jul 2010;19(7):1866-1870.
15. Gervasini G, San Jose C, Carrillo JA, Benitez J, Cabanillas A. GST polymorphisms interact with dietary factors to modulate lung cancer risk: study in a high-incidence area. *Nutr Cancer*. 2010;62(6):750-758.
16. Boeing H, Korfmann A, Bergmann MM. Recruitment procedures of EPIC-Germany. European Investigation into Cancer and Nutrition. *Ann Nutr Metab*. 1999;43(4):205-215.
17. Riboli E. The European Prospective Investigation into Cancer and Nutrition (EPIC): plans and progress. *J Nutr*. Jan 2001;131(1):170S-175S.
18. Bergmann MM, Bussas U, Boeing H. Follow-up procedures in EPIC-Germany--data quality aspects. European Prospective Investigation into Cancer and Nutrition. *Ann Nutr Metab*. 1999;43(4):225-234.
19. Rohrmann S, Becker N. Development of a short questionnaire to assess the dietary intake of heterocyclic aromatic amines. *Public Health Nutr*. Oct 2002;5(5):699-705.
20. Timofeeva M, Jager B, Rosenberger A, et al. A multiplex real-time PCR method for detection of GSTM1 and GSTT1 copy numbers. *Clin Biochem. oCancer Epidemiol Biomarkers Prev*. Nov 2010;19(11):2958-2968.
22. Steinbrecher A, Rohrmann S, Timofeeva M, Risch A, Jansen E, Linseisen J. Dietary glucosinolate intake, polymorphisms in selected biotransformation enzymes, and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev*. Jan 2010;19(1):135-143.
23. Di Paolo OA, Teitel CH, Nowell S, Coles BF, Kadlubar FF. Expression of cytochromes P450 and glutathione S-transferases in human prostate, and the potential for activation of heterocyclic amine carcinogens via acetyl-coA-, PAPS- and ATP-dependent pathways. *Int J Cancer*. Oct 20 2005;117(1):8-13.
24. Shaik AP, Jamil K, Das P. CYP1A1 polymorphisms and risk of prostate cancer: a meta-analysis. *Urol J*. Spring 2009;6(2):78-86.



25. Nelson CP, Kidd LC, Sauvageot J, et al. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione S-transferase P1. *Cancer Res.* Jan 1 2001;61(1):103-109.
26. Williams JA, Martin FL, Muir GH, Hewer A, Grover PL, Phillips DH. Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. *Carcinogenesis.* Sep 2000;21(9):1683-1689.
27. Iguchi T, Sugita S, Wang CY, Newman NB, Nakatani T, Haas GP. MnSOD genotype and prostate cancer risk as a function of NAT genotype and smoking status. *In Vivo.* Jan-Feb 2009;23(1):7-12.
28. Chang BL, Zheng SL, Isaacs SD, et al. Polymorphisms in the CYP1A1 gene are associated with prostate cancer risk. *Carcinogenesis.* Jun 2009;30(6):968-976.
30. Alaejos MS, Gonzalez V, Afonso AM. Exposure to heterocyclic aromatic amines from the consumption of cooked red meat and its effect on human cancer risk: a review. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* Jan 2008;25(1):2-24.
31. Mao C, Qiu LX, Zhan P, et al. MnSOD Val16Ala polymorphism and prostate cancer susceptibility: a meta-analysis involving 8,962 subjects. *J Cancer Res Clin Oncol.* Jul 2010;136(7):975-979.
32. Rohrmann S, Hermann S, Linseisen J. Heterocyclic aromatic amine intake increases colorectal adenoma risk: findings from a prospective European cohort study. *Am J Clin Nutr.*

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**Table 1:** Baseline characteristics of cases and controls in the EPIC-Heidelberg nested case-control study on PCa (n=563).

	Cases (n=203)	Controls (n=360)
	Mean (SD)	Mean (SD)
Age	57.91 (5.0)	57.93 (5.0)
BMI (kg/m <sup>2</sup> )	27.19 (3.4)	27.23 (3.7)
	N (%)	N (%)
<b>Education level</b>		
No/primary school	69 (34.0)	103 (28.7)
Vocational/other secondary school	69 (34.0)	118 (32.9)
University	65 (32.0)	138 (38.4)
<b>Smoking status</b>		
Never	80 (39.4)	123 (34.2)
Former	95 (46.8)	177 (49.2)
Current	28 (13.8)	60 (16.7)
<b>Positive family history of PCa</b>	14 (6.9)	7 (2.0)
<b>Advanced PCa</b>	98 (48.3)	
	Median (Interquartile range)	Median (Interquartile range)
<b>Energy Intake</b> (kcal/d)	1993 (1723-2371)	2076 (1732-2558)
<b>HCA Intake</b> (ng/day)		
Total PhIP	27.1 (11.4-79.1)	32.9 (12.0-86.2)
Total MeIQx	16.2 (8.0-34.1)	18.0 (8.0-37.3)
Total DiMeIQx	1.8 (0.8-4.2)	2.2 (0.8-4.5)
<b>Energy adjusted HCA Intake</b> (ng/1000 kcal)		
Total PhIP	13.1 (6.0-37.1)	15.8 (6.0-40.3)
Total MeIQx	7.8 (4.0-16.2)	8.5 (4.1-16.8)
Total DiMeIQx	0.9 (0.4-2.0)	1.1 (0.4-2.3)
<b>Meat intake by degree of browning</b> (g/day)		
Very light/light	22.7 (9.9-37.4)	23.7 (9.8-44.2)

Medium/ dark	10.7 (2.5-28.3)	13.3 (1.4-33.8)
<b>Calcium</b> (g/d)	0.8 (0.5-0.9)	0.8 (0.6-1.0)
<b>Tomatoes, tomato products</b> (g/d)	2.0 (1.0-3.0)	2.0 (1.0-3.0)
<b>Red meat</b> (g/d)	31.3 (20.7-50.0)	34.2 (19.7-58.6)
<b>Processed meat</b> (g/d)	51.9 (32.4-71.2)	56.3 (32.4-82.5)
<b>White meat</b> (g/d)	8.3 (4.3-15.5)	9.6 (3.9-17.2)
<b>Alcohol</b> (g/d)	21.8 (9.4-39.3)	18.4 (6.6-34.9)

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**Table 2:** Allele frequencies of selected genetic variants in PCa cases and controls in the EPIC-Heidelberg nested case-control study (n=563).

Genotype	All	Cases (%)	Controls (%)
<b><i>GSTMI</i></b>			
del/del	293 (52.04)	105 (51.72)	188 (52.22)
pres/del	220 (39.08)	81 (39.90)	139 (38.61)
pres/pres	50 (8.88)	17 (8.37)	33 (9.17)
HWE§	0.35		0.32
<b><i>GSTT1</i></b>			
del/del	99 (17.58)	35 (17.24)	64 (17.78)
pres/del	285 (50.62)	100 (49.26)	185 (51.39)
pres/pres	179 (31.79)	68 (33.50)	111 (30.83)
HWE§	0.43		0.37
<b><i>NQO1</i> (rs1800566)</b>			
CC	382 (67.85)	132 (65.02)	250 (69.44)
CT	158 (28.06)	66 (32.51)	92 (25.56)
TT	23 (4.09)	5 (2.46)	18 (5.00)
HWE§	0.2		0.02
<b><i>GSTAI</i> (rs3957357)</b>			
GG	166 (29.48)	62 (30.54)	104 (28.89)
GA	278 (49.38)	100 (49.26)	178 (49.44)
AA	119 (21.14)	41 (20.20)	78 (21.67)
HWE§	0.9		0.91
<b><i>GSTP1</i> (rs1695)</b>			
AA	258 (45.83)	100 (49.26)	158 (43.89)
AG	249 (44.23)	81 (39.90)	168 (46.67)
GG	56 (9.95)	22 (10.84)	34 (9.44)
HWE§	0.72		0.26
<b><i>GSTP1</i> (rs1138272)</b>			
CC	471 (83.66)	299 (83.06)	172 (84.73)
CT	91 (16.16)	61 (16.94)	30 (14.78)
TT	1 (0.18)	0 (0.00)	1 (0.49)
HWE§	0.11		0.8
<b><i>MnSOD</i> (rs4880)</b>			
CC	143 (25.40)	53 (26.11)	90 (25.00)
CT	290 (51.51)	100 (49.26)	190 (52.78)
TT	130 (23.90)	50 (24.63)	80 (22.22)
HWE§	0.47		0.28
<b><i>CYP1A1</i>*4 (rs1799814)</b>			
CC	512 (90.94)	177 (87.19)	335 (93.06)
CA	51 (9.06)	26 (12.81)	25 (6.94)
HWE§	0.26		0.49
<b><i>CYP1A1</i> *2A (rs4646903)</b>			
TT	470 (83.47)	165 (81.28)	305 (84.72)
TC	89 (15.81)	35 (17.24)	54 (15.00)
CC	4 (0.71)	3 (1.48)	1 (0.28)
HWE§	0.92		0.37
<b><i>GPXI</i> (rs1050450)</b>			

CC	302 (54.22)	104 (51.49)	198 (55.77)
CT	204 (36.62)	82 (40.59)	122 (34.37)
TT	51 (9.16)	16 (7.92)	35 (9.86)
HWE§	0.74		0.02
<b><i>GPX4</i> C718T</b> (rs713041)			
CC	171 (30.54)	64 (31.84)	107 (29.81)
CT	266 (47.50)	91 (45.27)	175 (48.75)
TT	123 (21.96)	46 (22.89)	77 (21.45)
HWE§	0.31		0.73

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Abbreviations: HWE, Hardy-Weinberg equilibrium;

§ P-value of  $\chi^2$  test.

Genotype	Cases/Controls	50 ng/day increments of energy-adjusted PhIP intake		10 ng/day increments of energy-adjusted MeIQx intake		1 ng/day increments of energy-adjusted DiMeIQx intake	
		OR <sup>1</sup> (95%CI)	P <sub>i</sub>	OR <sup>1</sup> (95%CI)	P <sub>i</sub>	OR <sup>1</sup> (95%CI)	P <sub>i</sub>
<b>GSTM1</b>							
pres/pres	17/33	0.71 (0.22-2.33)		0.96 (0.52-1.79)		1.01 (0.75-1.36)	
pres/del	81/139	0.70 (0.49-1.00)		0.85 (0.68-1.07)		0.93 (0.79-1.08)	
del/del	105/188	0.95 (0.82-1.09)	0.1	1.05 (0.97-1.16)	0.12	1.00 (0.93-1.07)	0.54
≥1 del	186/327	0.89 (0.77-1.02)	0.57	1.01 (0.93-1.10)	0.7	0.99 (0.93-1.05)	0.96
<b>GSTT1</b>							
pres/pres	68/111	0.70 (0.49-0.99)		0.78 (0.60-1.01)		0.79 (0.63-0.98)	
pres/del	100/185	0.99 (0.83-1.20)		1.09 (0.98-1.20)		1.01 (0.93-1.09)	
del/del	35/64	0.88 (0.64-1.21)	0.38	0.96 (0.73-1.27)	0.18	1.00 (0.83-1.21)	0.11
≥1 del	135/249	0.95 (0.82-1.11)	0.14	1.07 (0.97-1.17)	0.05	1.01 (0.95-1.08)	0.05
<b>GSTT1/GSTM1</b>							
0 or 1 del	44/69	0.55 (0.30-1.02)		0.73 (0.50-1.05)		0.69 (0.49-0.98)	
2 del	73/141	0.76 (0.55-1.07)		0.85 (0.66-1.10)		0.95 (0.87-1.04)	
3 or 4 del	86/150	1.01 (0.86-1.19)	0.03	1.09 (0.98-1.21)	0.01	1.02 (0.95-1.10)	0.03
<b>NQO1</b> (rs1800566)							
CC	132/250	0.95 (0.82-1.10)		1.00 (0.88-1.13)		1.01 (0.92-1.11)	
CT	66/92	0.74 (0.54-1.02)		1.03 (0.92-1.16)		0.58 (0.24-1.44)	
TT	Mai 18	1.16 (0.14-9.40)	0.16	0.54 (0.12-2.51)	0.88	NA	0.33
≥1 T	71/110	0.74 (0.54-1.01)	0.16	1.03 (0.92-1.15)	0.88	0.94 (0.86-1.03)	0.4
<b>GSTA1</b> (rs3957357)							
GG	62/104	0.77 (0.56-1.07)		1.02 (0.90-1.15)		0.99 (0.90-1.11)	
GA	100/178	0.92 (0.76-1.11)		1.00 (0.86-1.15)		0.99 (0.89-1.10)	
AA	41/78	0.87 (0.65-1.18)	0.66	0.93 (0.74-1.16)	0.58	0.91 (0.78-1.06)	0.38
≥1 A	141/256	0.90 (0.77-1.06)	0.5	0.98 (0.87-1.10)	0.6	0.96 (0.89-1.04)	0.47
<b>GSTP1</b> (rs1695)							
AA	100/158	0.90 (0.77-1.06)		1.02 (0.91-1.14)		1.04 (0.95-1.14)	
AG	81/168	0.88 (0.66-1.17)		0.95 (0.78-1.15)		0.91 (0.79-1.05)	
GG	22/34	0.77 (0.46-1.26)	0.76	1.02 (0.87-1.20)	0.87	0.94 (0.83-1.06)	0.23
≥1 G	103/202	0.85 (0.65-1.09)	0.79	1.00 (0.89-1.13)	0.82	0.93 (0.85-1.01)	0.1
<b>GSTP1</b> (rs1138272)							
CC	172/299	0.88 (0.76-1.02)		0.98 (0.89-1.09)		1.02 (0.95-1.10)	
CT	30/61	0.87 (0.55-1.37)	0.96	1.10 (0.92-1.32)	0.37	0.89 (0.77-1.03)	0.17
TT	0/0	NA		NA		NA	
<b>MnSOD</b> (rs4880)							
CC	53/90	0.77 (0.54-1.10)		1.10 (0.96-1.26)		1.12 (0.97-1.29)	
CT	100/190	0.93 (0.79-1.09)		0.97 (0.85-1.11)		1.04 (0.94-1.14)	
TT	50/80	0.83 (0.58-1.19)	0.72	0.93 (0.73-1.20)	0.2	0.83 (0.66-1.04)	0.02
≥1 T	150/270	0.91 (0.78-1.06)	0.42	0.96 (0.86-1.08)	0.19	0.96 (0.90-1.03)	0.08
<b>CYP1A1</b> (rs1799814)							
CC	177/335	0.86 (0.74-1.01)		0.97 (0.87-1.08)		1.01 (0.94-1.09)	
CA	26/25	0.99 (0.68-1.46)	0.62	1.11 (0.90-1.37)	0.26	0.95 (0.84-1.07)	0.29
<b>CYP1A1</b> (rs4646903)							

Genotype	Cases/Controls	50 ng/day increments of energy-adjusted PhIP intake		10 ng/day increments of energy-adjusted MeIQx intake		1 ng/day increments of energy-adjusted DiMeIQx intake	
		OR <sup>1</sup> (95%CI)	P <sub>i</sub>	OR <sup>1</sup> (95%CI)	P <sub>i</sub>	OR <sup>1</sup> (95%CI)	P <sub>i</sub>
<b>GSTM1</b>							
pres/pres	17/33	0.71 (0.22-2.33)		0.96 (0.52-1.79)		1.01 (0.75-1.36)	
pres/del	81/139	0.70 (0.49-1.00)		0.85 (0.68-1.07)		0.93 (0.79-1.08)	
del/del	105/188	0.95 (0.82-1.09)	0.1	1.05 (0.97-1.16)	0.12	1.00 (0.93-1.07)	0.54
≥1 del	186/327	0.89 (0.77-1.02)	0.57	1.01 (0.93-1.10)	0.7	0.99 (0.93-1.05)	0.96
<b>GSTT1</b>							
pres/pres	68/111	0.70 (0.49-0.99)		0.78 (0.60-1.01)		0.79 (0.63-0.98)	
pres/del	100/185	0.99 (0.83-1.20)		1.09 (0.98-1.20)		1.01 (0.93-1.09)	
del/del	35/64	0.88 (0.64-1.21)	0.38	0.96 (0.73-1.27)	0.18	1.00 (0.83-1.21)	0.11
≥1 del	135/249	0.95 (0.82-1.11)	0.14	1.07 (0.97-1.17)	0.05	1.01 (0.95-1.08)	0.05
<b>GSTT1/GSTM1</b>							
0 or 1 del	44/69	0.55 (0.30-1.02)		0.73 (0.50-1.05)		0.69 (0.49-0.98)	
2 del	73/141	0.76 (0.55-1.07)		0.85 (0.66-1.10)		0.95 (0.87-1.04)	
3 or 4 del	86/150	1.01 (0.86-1.19)	0.03	1.09 (0.98-1.21)	0.01	1.02 (0.95-1.10)	0.03
<b>NQO1</b> (rs1800566)							
CC	132/250	0.95 (0.82-1.10)		1.00 (0.88-1.13)		1.01 (0.92-1.11)	
CT	66/92	0.74 (0.54-1.02)		1.03 (0.92-1.16)		0.58 (0.24-1.44)	
TT	Mai 18	1.16 (0.14-9.40)	0.16	0.54 (0.12-2.51)	0.88	NA	0.33
≥1 T	71/110	0.74 (0.54-1.01)	0.16	1.03 (0.92-1.15)	0.88	0.94 (0.86-1.03)	0.4
<b>GSTA1</b> (rs3957357)							
GG	62/104	0.77 (0.56-1.07)		1.02 (0.90-1.15)		0.99 (0.90-1.11)	
GA	100/178	0.92 (0.76-1.11)		1.00 (0.86-1.15)		0.99 (0.89-1.10)	
AA	41/78	0.87 (0.65-1.18)	0.66	0.93 (0.74-1.16)	0.58	0.91 (0.78-1.06)	0.38
≥1 A	141/256	0.90 (0.77-1.06)	0.5	0.98 (0.87-1.10)	0.6	0.96 (0.89-1.04)	0.47
<b>GSTP1</b> (rs1695)							
AA	100/158	0.90 (0.77-1.06)		1.02 (0.91-1.14)		1.04 (0.95-1.14)	
AG	81/168	0.88 (0.66-1.17)		0.95 (0.78-1.15)		0.91 (0.79-1.05)	
GG	22/34	0.77 (0.46-1.26)	0.76	1.02 (0.87-1.20)	0.87	0.94 (0.83-1.06)	0.23
≥1 G	103/202	0.85 (0.65-1.09)	0.79	1.00 (0.89-1.13)	0.82	0.93 (0.85-1.01)	0.1
<b>GSTP1</b> (rs1138272)							
CC	172/299	0.88 (0.76-1.02)		0.98 (0.89-1.09)		1.02 (0.95-1.10)	
CT	30/61	0.87 (0.55-1.37)	0.96	1.10 (0.92-1.32)	0.37	0.89 (0.77-1.03)	0.17
TT	0/0	NA		NA		NA	
<b>MnSOD</b> (rs4880)							
CC	53/90	0.77 (0.54-1.10)		1.10 (0.96-1.26)		1.12 (0.97-1.29)	
CT	100/190	0.93 (0.79-1.09)		0.97 (0.85-1.11)		1.04 (0.94-1.14)	
TT	50/80	0.83 (0.58-1.19)	0.72	0.93 (0.73-1.20)	0.2	0.83 (0.66-1.04)	0.02
≥1 T	150/270	0.91 (0.78-1.06)	0.42	0.96 (0.86-1.08)	0.19	0.96 (0.90-1.03)	0.08
<b>CYP1A1</b> (rs1799814)							
CC	177/335	0.86 (0.74-1.01)		0.97 (0.87-1.08)		1.01 (0.94-1.09)	
CA	26/25	0.99 (0.68-1.46)	0.62	1.11 (0.90-1.37)	0.26	0.95 (0.84-1.07)	0.29
<b>CYP1A1</b> (rs4646903)							

TT	165/305	0.88 (0.77-1.02)		1.02 (0.94-1.11)		1.00 (0.94-1.06)	
TC	35/54	1.00 (0.59-1.71)		0.88 (0.60-1.29)		0.94 (0.73-1.20)	
CC	03. Jan	NA	0.75	NA	0.4	NA	0.46
≥1 C	38/55	0.99 (0.58-1.68)	0.72	0.88 (0.60-1.30)	0.39	0.93 (0.73-1.19)	0.48
<b>GPXI</b> (rs1050450)							
CC	104/198	0.86 (0.71-1.04)		1.04 (0.94-1.15)		0.97 (0.89-1.05)	
CT	82/122	0.80 (0.59-1.09)		0.88 (0.72-1.07)		1.01 (0.92-1.11)	
TT	16/35	2.17 (1.00-4.61)	0.17	1.27 (0.93-1.74)	0.87	1.03 (0.80-1.33)	0.55
≥1 T	98/157	0.93 (0.76-1.13)	0.65	0.97 (0.84-1.13)	0.37	1.01 (0.93-1.11)	0.51
<b>GPX4 C718T</b> (rs713041)							
CC	64/107	0.77 (0.55-1.09)		1.03 (0.93-1.15)		1.10 (0.99-1.22)	
CT	91/175	0.89 (0.75-1.05)		1.00 (0.86-1.15)		0.93 (0.82-1.07)	
TT	46/77	0.99 (0.70-1.41)	0.29	0.98 (0.72-1.31)	0.64	0.90 (0.77-1.04)	0.03
≥1 T	137/252	0.90 (0.78-1.04)	0.39	0.99 (0.87-1.12)	0.66	0.92 (0.83-1.01)	0.01

Abbreviation: P<sub>i</sub>, P-value for test of interaction between HCA intake and genotype; NA, not available.

1: OR calculated by unconditional logistic regression adjusted for matching variables (time of recruitment and 5-year age group) and family history of prostate cancer.

**Table 4:** Relative risk (OR, 95%CI) of PCa with increasing meat intake by degree of browning in strata of selected genotypes in the EPIC-Heidelberg nested case-control study on PCa (n=563).

Genotype	Cases/Controls	10g/day increments of meat consumed dark		10g/day increments of meat consumed light	
		OR <sup>1</sup> (95%CI)	P <sub>i</sub>	OR <sup>1</sup> (95%CI)	P <sub>i</sub>
<b><i>GSTMI</i></b>					
pres/pres	17/33	0.85 (0.62-1.16)		0.92 (0.74-1.16)	
pres/del	81/139	1.01 (0.91-1.13)		0.97 (0.88-1.07)	
del/del	105/188	0.95 (0.87-1.04)	0.97	0.94 (0.85-1.04)	0.63
≥1 del	186/327	0.98 (0.91-1.04)	0.23	0.96 (0.89-1.02)	0.85
<b><i>GSTTI</i></b>					
pres/pres	68/111	0.86 (0.75-0.98)		0.90 (0.81-1.01)	
pres/del	100/185	1.03 (0.94-1.12)		0.95 (0.86-1.06)	
del/del	35/64	0.92 (0.76-1.11)	0.25	1.08 (0.93-1.25)	0.1
≥1 del	135/249	1.01 (0.94-1.09)	0.07	0.99 (0.91-1.08)	0.19
<b><i>GSTTI/GSTMI</i></b>					
0 or 1 del	44/69	0.88 (0.74-1.04)		0.87 (0.75-1.00)	
2 del	73/141	0.97 (0.86-1.08)		1.01 (0.92-1.12)	
3 or 4 del	86/150	0.99 (0.90-1.09)	0.24	0.96 (0.86-1.08)	0.43
<b><i>NQOI</i> (rs1800566)</b>					
CC	132/250	0.99 (0.90-1.08)		0.97 (0.90-1.05)	
CT	66/92	0.95 (0.85-1.06)		0.91 (0.80-1.04)	
TT	Mai 18	0.73 (0.43-1.24)	0.18	1.11 (0.77-1.60)	0.74
≥1 T	71/110	0.93 (0.84-1.03)	0.33	0.92 (0.82-1.04)	0.59
<b><i>GSTAI</i> (rs3957357)</b>					
GG	62/104	0.94 (0.82-1.08)		0.88 (0.75-1.03)	
GA	100/178	0.98 (0.89-1.07)		0.96 (0.89-1.04)	
AA	41/78	0.98 (0.86-1.13)	0.49	1.05 (0.90-1.23)	0.12
≥1 A	141/256	0.98 (0.91-1.06)	0.54	0.98 (0.91-1.05)	0.17
<b><i>GSTPI</i> (rs1695)</b>					
AA	100/158	0.98 (0.90-1.07)		0.95 (0.87-1.04)	
AG	81/168	0.98 (0.88-1.08)		0.92 (0.82-1.03)	
GG	22/34	0.82 (0.62-1.08)	0.3	1.14 (0.89-1.45)	0.54
≥1 G	103/202	0.95 (0.86-1.05)	0.53	0.95 (0.86-1.05)	0.9
<b><i>GSTPI</i> (rs1138272)</b>					
CC	172/299	0.98 (0.91-1.05)		0.95 (0.88-1.02)	
CT	30/61	0.89 (0.72-1.09)	0.31	1.01 (0.87-1.18)	0.38
TT	0/0	NA		NA	
<b><i>MnSOD</i> (rs4880)</b>					
CC	53/90	1.03 (0.91-1.16)		1.08 (0.95-1.23)	
CT	100/190	1.02 (0.93-1.12)		0.83 (0.74-0.92)	
TT	50/80	0.74 (0.60-0.91)	0.02	1.13 (0.98-1.30)	0.81
≥1 T	150/270	0.95 (0.87-1.03)	0.33	0.92 (0.85-0.99)	0.04
<b><i>CYP1A1</i> (rs1799814)</b>					
CC	177/335	0.94 (0.87-1.01)		0.96 (0.89-1.03)	
CA	26/25	1.06 (0.90-1.24)	0.11	0.92 (0.74-1.16)	0.88
<b><i>CYP1A1</i> (rs4646903)</b>					
TT	165/305	0.99 (0.92-1.06)		0.97 (0.90-1.04)	



TC	35/54	0.82 (0.64-1.06)		0.87 (0.70-1.09)	
CC		NA	0.21	NA	0.42
≥1 C	38/55	0.84 (0.66-1.06)	0.16	0.88 (0.71-1.08)	0.49
<b>GPXI</b> (rs1050450)					
CC	104/198	0.94 (0.85-1.03)		0.93 (0.85-1.02)	
CT	82/122	0.99 (0.90-1.09)		1.01 (0.91-1.11)	
TT		1.06 (0.79-1.42)	0.31	0.89 (0.66-1.20)	0.52
≥1 T	98/157	1.00 (0.91-1.10)	0.46	0.99 (0.91-1.09)	0.26
<b>GPX4 C718T</b> (rs713041)					
CC	64/107	0.95 (0.84-1.08)		0.99 (0.89-1.09)	
CT	91/175	1.00 (0.91-1.09)		0.96 (0.87-1.06)	
TT	46/77	0.89 (0.76-1.04)	0.67	0.88 (0.74-1.05)	0.27
≥1 T	137/252	0.97 (0.89-1.09)	0.99	0.94 (0.86-1.02)	0.55

Abbreviation: P<sub>i</sub>, P-value for test of interaction between HCA intake and genotype; NA, not available.

1: OR calculated by unconditional logistic regression adjusted for matching variables (time of recruitment and 5-year age group), family history of prostate cancer, and energy-intake (kJ/d).